Breeding Studies in Potatoes Containing High Concentrations of Anthocyanins

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ABSTRACT

Studies of the breeding behavior of clones containing high levels of anthocyanins were conducted. Red-fleshed clones appeared in proportions suggesting multigenic control of degree of pigmentation. Red-fleshed and purple-fleshed clones were always accompanied by red and purple skin, respectively. Red flesh ranged from partial pigmentation to complete pigmentation represented by pigment present in all tuber tissues. Percentage of completely red-fleshed progeny was 14.5% and 4.1% in red x red crosses vs red x white (or the reciprocal), respectively. Purple-fleshed progeny were obtained from red x white crosses where the white-fleshed parent harbored the *P* pigment gene in juxtaposition with the nulliplex recessive state of the *I* gene (i.e., *iiii*), which suppressed expression. Total anthocyanin ranged from 6.9 to 35 mg per 100 g fresh weight in the red-fleshed and 5.5 to 17.1 in the purple-fleshed clones. Red-fleshed clones contained predominantly acylated glycosides of pelargonidin while the purple-fleshed clones contained predominantly acylated glycosides of petunidin and peonidin. Oxygen Radical Absorbance Capacity and Ferrous Reducing Ability of Plasma revealed that the antioxidant levels in the red or purple-fleshed potatoes were two to three times higher than white-fleshed potato.

RESUMEN

Se llevaron a cabo estudios de mejoramiento sobre el comportamiento de clones de papa con niveles altos de antocianinas. Los clones de pulpa roja mostraron esta característica en mayor proporción, lo que sugiere un control multigénico del grado de pigmentación. Los clones de pulpa roja y los de pulpa morada estuvieron siempre acompañados de piel roja y piel morada respectivamente. La pulpa roja varió desde una pigmentación parcial a una pigmentación completa, en el cual el pigmento se encontraba presente en todos los tejidos del tubérculo. El porcentaje en una progenie de pulpa roja completa fue de 14.5% y 4.1% en los cruzamientos rojo x rojo vs. rojo x blanco (o recíproco) respectivamente. La progenie de pulpa morada se obtuvo de cruzamientos rojo x blanco, donde el progenitor de pulpa blanca albergaba el gen del pigmento morado *P* en yuxtaposición con el estado recesivo nuliplex del gen inhibidor *I*, el cual suprimió la expresión. La antocianina total varió de 6.9 a 35mg por 100g de peso fresco en los clones de pulpa roja y de 5.5 a 17.1 mg en los de pulpa morada. Los clones de pulpa roja contenían predominantemente glicósidos acilados de pelargonidina, mientras que los clones morados contenían predominantemente glicósidos acilados de petunidina y pelargonidina. La Capacidad de Absorbencia del Radical Oxigeno y la Habilidad del Plasma para la Reducción del Hierro revelaron que los niveles de antioxidante en papas de pulpa roja o morada fueron de dos a tres veces mayores que en las papas de pulpa blanca.

INTRODUCTION

The natural variation of cultivated potato germplasm includes types that are red and purple pigmented due to the presence of flavonoids in the skin and/or flesh. Among the many flavonoids that may be found in potato tubers are the anthocyanins. A series of single genes control presence and

Accepted for publication 7 May 2003.
ADDITIONAL KEY WORDS: Antioxidants, pelargonidin, petunidin, peonidin, ORAC, FRAP.

absence of red and blue pigments. Although different genetic systems controlling pigment expression have been identified for diploid cultivated vs tetraploid cultivated potatoes, (Dodds and Long 1955, 1956; Lunden 1960), De Jong (1991) and Van Eck and coworkers (1994) have argued that the genes appear to be syntenic and should be regarded as belonging to one genome. The symbol *D* denotes a single gene controlling synthesis of red pigment, located on chromosome 2: the symbol *P* stands for a single gene on chromosome 11 controlling blue pigment synthesis, while *I*, of undetermined location, epistatically controls presence and absence of tuber skin and flesh pigmentation even when *P* and *D* are present. Gebhardt et al. (1989) reported a locus controlling purple skin color, *Psc*, on chromosome 4. The single gene *Pf*, linked to *I*, determines whether pigment is present beyond the periderm in the interior tissues of the tuber (DeJong 1987, 1991; Van Eck et al. 1994). The pigments have been determined to be varied types of acylated anthocyanidin glycosides (Harbourne 1960; Rodriguez-Saona et al. 1998). The gene *Ac* is imputed to control acylation of anthocyanins. Diploid cultivated potatoes display both acylated and non-acylated forms while only acylated anthocyanins are present in the tetraploid cultivars (Swaminathan and Howard 1953). Red- and purple-skinned potato varieties are not uncommon. Potatoes with varying distributions of anthocyanin in the flesh, appearing as rings and arcs and radiating stars are found in the native cultivars of the Andes and rarely among varieties in the rest of the world. Solidly colored red or purple tuber flesh is unusual throughout the world, but occurs as rare segregants in certain red- and purple-skinned breeding populations and a few pigmented flesh types are available commercially as specialty potatoes, e.g., cvs. Cranberry Red, All Blue.

Anthocyanins have been found to have antioxidant properties in fruits and vegetables and potatoes. Diets rich in antioxidant flavonoids and carotenoids have been associated with a lower incidence of atherosclerotic heart disease, certain cancers, macular degeneration and severity of cataracts (Cao et al. 1998, 1999; Hertog et al. 1993; Knekt et al. 1996; Kruezer 2001; Wang et al. 1999). Hence the possible nutritive value of anthocyanins in potato invites studies into the breeding behavior and expression of these pigments in tetraploid populations. The enhancement of anthocyanins in potato provides the opportunity to radically change certain aspects of the food value of potato and processed foods made from potato.

In particular, the antioxidant capacity conferred by anthocyanins is of interest. Oxygen Radical Absorbance Capacity (ORAC) is a measure of the capacity of an antioxidant to delay oxidation of a target molecule. In ORAC this is measured by detecting the loss of luminescence of β-Phycoerythrin (PE) due to oxidation. The loss of PE fluorescence in the presence of free radicals is an index of oxidative damage to the protein. The assay uses 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) as a free radical–generating system and an area under curve technique for quantitation of antioxidant capacity. AAPH undergoes spontaneous decomposition and produces peroxyl radicals with a rate dependent on temperature. Thus, the ORAC assay measures the capacity of an antioxidant to directly quench free radicals. The ORAC method is considered to have the advantage that it combines both inhibition percentage and the length of inhibition time of free radical action by an antioxidant into a single quantity (Cao and Prior 1998).

The Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain 1996) is a measure of the ferric-to-ferrous iron reduction followed by the formation of colored ferrous-tripyridyltriazine complex in the presence of antioxidants. It is easier and less expensive to carry out than ORAC, but presents only a single time point percentage inhibition of oxidation.

MATERIALS AND METHODS

Genetic stocks were obtained from breeding populations designed to produce red-skinned varieties, white-skinned varieties, and long russets. The origin of parents used in the crosses is set forth in Table 1. Crosses were made according to the standard procedures outlined by Plaisted (1980). True seed was extracted from berries, air-dried on paper towels, and treated by soaking in an aqueous solution of 1,500 ppm gibberellic acid (Sigma, St. Louis, MO, USA). Tubers for extraction of anthocyanins were produced by growing plants for four months in plastic pots (8 in. dia) using Sunshine Mix #1 as the soil mix, under halide lights providing 14-h daylength of 15,000 photosynthetic photon flux density umol photons/m^2 per second.

Extraction of pigments followed the procedure described by Giusti and Wrolstad (1996) with modifications. Potato tubers (ca 50 g) were diced in cubes approximately 0.5 cm a side. Approximately 10 g of diced potatoes were placed in a Waring blendor, liquid nitrogen was added, and the mixture ground until pulverized. Ten grams of powder were placed in a liquid-nitrogen-cooled 25-mL screw-cap glass tube in equal parts (w/w) acetone and water. The mixture was sonicated 10 to 20 min until it reached room temperature, centrifuged at

TABLE 1—*Parents used in crossing, characteristics and origin.*

Parent	Characteristics	Origin
NDOP5847-1	Red skin, completely red flesh	Breeding clone selected from North Dakota State University Cross 4255-3R x cv Bison.
N40-2	Red skin, completely red flesh	Breeding clone from Cornell University, Ithaca, NY.
Cranberry Red	Red skin, pink flesh	Heirloom variety obtained from Wood Prairie Farms, Bridgewater, Maine
A89875-5	White skin, white flesh	Corky ringspot resistant breeding clone, USDA/ARS, Aberdeen, ID
A77715-6	White skin, white flesh, purple-tipped sprouts	Corky ringspot resistant breeding clone, USDA/ARS, Aberdeen, ID
A8469-5	Oblong shape, russet skin, white flesh	Breeding clone-USDA/ARS, Aberdeen, ID
Andover	Named variety, white skin, white flesh	Cornell University Potato Breeding Program, Ithaca New York
Kanona	Named variety, white skin, white flesh	Cornell University Potato Breeding Program, Ithaca New York
Pike	Named variety, white skin, white flesh	Cornell University Potato Breeding Program, Ithaca New York
Abnaki	Named variety, white skin, white flesh, purple sprouts	Maine Variety, Presque Isle, Maine; seed source USDA/ARS, Aberdeen, ID
Serrana	Named variety, white skin, white flesh, purple-tipped sprouts	Argentine variety, INTA, Balcarce, Argentina. Seed source, USDA/ARS, Aberdeen, ID
Atlantic	Named variety, white skin, white flesh	USDA/ARS variety, Beltsville, MD. Seed source-USDA/ARS, Aberdeen, ID
AWN86514-2	Russet skin, white flesh, late blight resistant	Breeding clone, USDA/ARS, Prosser, WA
PA96RR1-193	Red skin, red flesh	Breeding clone, USDA/ARS, Prosser, WA. (Fontenot x 3261-5R)
PA96RR1-220	Red skin, red flesh	Breeding clone, USDA/ARS, Prosser, WA, (Fontenot x 3261-5R)

3800 rpm for 5 min, and the supernatant was collected. The extraction above was repeated on the pellet using 70% acetone-water mixture. Both supernatants were combined and placed in hot water bath sufficiently warm to boil acetone for 10 min to deactivate polyphenol oxidase activity during which acetone was added to maintain volume. The acetone solution was partitioned with 1.5 to 3 volumes of chloroform in a separatory funnel retaining the aqueous phase. The acetone was removed on a Buchi rotovapor at 40 C and residue was dissolved in a known volume of distilled water. Two replications of each potato genotype were measured.

Monomeric anthocyanin content was determined using a pH-differential method (Giusti and Wrolstad 2001). A Shimadzu 300 UV spectrophotometer with 1-cm path-length cells was used for spectral measurements at 420, 510, and 700 nm. Pigment content was calculated as pelargonidin-3-glucoside (Pg-3-glu), using an extinction coefficient of 31,600L cm^{-1} and molecular weight of 433.2 g mol^{-1} (Giusti and Wrolstad 2001).

Anthocyanin Purification

The aqueous extract was passed through a C-18 mini-column (high load C-18 tube). Twenty-milliliter capacity and 5 g sorbent weight (Alltech Assoc., Inc. IL, USA), previously activated with methanol followed by 0.01% aqueous HCl (Giusti and Wrolstad 1996). Anthocycanins and phenolic acids were adsorbed onto the mini-column, sugars and acids were eluted with two volumes of 0.01% aqueous HCl and anthocyanins were recovered with methanol containing 0.01% HCl (w/v). The methanol was evaporated using a Buchi rotovapor at 40 C and the pigments were dissolved in deionized water containing 0.01% HCl.

HPLC Analysis

An analytical High Performance Liquid Chromatograph (HPLC) Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector and Gateway 2000 P5-90 computer with a Hewlett Packard HPLC2D Chemstation software was used. The following steps were applied to achieve separation and detection: System I—Polymer Labs PLRV P-S column (5 micron) 250 x 4.6 mm i.d. (Polymer Labs, Amherst, MA, USA), fitted with a Polymer Labs 15 x 4.6 mm i.d. guard column (Solvent A: 100% HPLC grade acetonitrile; B: 4% phosphoric acid [aqueous]); System II—ODS C-18 column (5 micron), 250 x 4.6 i.d. (Poly LC, Inc., Columbia, MD, USA), fitted with a 10 x 4.6 mm i.d. Sherisorb ODS-2 micro guard column (Alltech, Deerfield, IL, USA) (Solvent A: 100% HPLC grade acetonitrile, B: 1% phosphoric acid, 10 % acetic acid, 5% acetonitrile and water); System III—Supelcosil LC-18 column (5 micron) 250 x 4.6 mm i.d. (Supelco Inco. Bellefonte, PA, USA), fitted with a 10 x 4.6 mm Spherisorb ODS-2 micro guard column (Alltech, Deerfield, IL, USA), (Solvent A: 100% HPLC grade methanol; B: 0.07 M KH_2PO_4 [aqueous] adjusted to pH 2.5 with phosphoric acid). All analytical systems were run at 1 mL/min and an injection volume of 50 μL. Solvents and samples were filtered through a 0.45 micron Millipore filter type

HA (Millipore Corp., Bedford, MA). Percent composition was determined from the average of two replicates derived from separate extractions.

Mass Spectroscopy of Potato Anthocyanins

Anthocyanin extracts were saponified and hydrolyzed following procedure of Durst and Wrolstad (2001). Low-resolution mass spectroscopy was performed using electro-spray ionization (ESMS). The instrument was a Perkin-Elmer SCI-EX API III+Mass Spectrometer equipped with an ION Spray source ISV=4700, orifice voltage of 80 and loop injection. Partially purified potato anthocyanin extracts, saponified and hydrolyzed derivatives, and HPLC purified anthocyanins were injected directly into the system.

Antioxidant Measurements

Determination of Antioxidant Capacity—Antioxidant capacity was determined by ORAC and FRAP assays at the Linus Pauling Institute, Oregon State University. The ORAC assay was performed as described by Cao et al. (1993) and adapted for use in a 96-well microplate fluorometer (model Cytofluor 4000, PerSeptive Biosystems, Framingham, MA, USA). ORAC values, derived from triplicate analyses, are expressed as mmoles Trolox equivalents (TE) per gram of fresh-frozen fruit. Trolox is a water-soluble tocopherol analogue used as a reference compound for antioxidant capacity. The FRAP assay (Benzie and Strain 1996) was adapted for use in a 96-well microplate spectrophotometer (ThermoMax, Molecular Devices, Foster City, CA, USA). FRAP values, derived from triplicate analyses, are expressed as mmoles ferric iron reduced per gram of fresh-frozen fruit.

The Oxygen Radical Absorbance Capacity (ORAC) depends on the detection of chemical damage to Beta or R-phycoerythrin (RE) through the decrease in the fluorescence emission. The values are expressed in micromoles Trolox equivalents per gram fresh weight (Cao et al. 1993, 1995). The technique used here followed the procedure of Cao and coworkers (1995) without modification.

The Ferric Reducing Ability of Plasma (FRAP) method of Benzie and Strain (1996) was employed without modification expressing the antioxidant activity, as with ORAC as micromoles Trolox equivalent per gram fresh weight.

RESULTS AND DISCUSSION

Genetic materials providing the red-fleshed trait were identified in a clonal selection NDOP5847-1, derived originally from the a cross made by Dr. R. Johanssen at North Dakota State University, the breeding clone N40-2, kindly provided by Dr. R. L. Plaisted, of the Deparment of Plant Breeding and Biometry, Cornell University, and the specialty variety Cranberry Red. The genetic factor for purple pigment in the flesh was derived from A77715-6 and cv. Serrana, which are, interestingly, white fleshed. They must possess the *P* gene juxtaposed to the recessive state of gene *I*, i.e. *iiii*. The derivation of purple-fleshed trait from these two parents, an unintended outcome, revealed the presence of the unexpressed *P* as a result of observing purple fleshed progeny.

Crossing

The numbers and percentages of phenotypes obtained from crosses involving red flesh are presented in Table 2. There are four red-fleshed x red-fleshed crosses and eleven crosses where only one parent is red fleshed. Phenotypes were categorized as having partially red flesh (RPR = red skin, partially red flesh) or completely red flesh (RCR = red skin, completely red flesh), the latter term denoting that some level of pigmentation is distributed in all the tissues of the tuber. Presence of red pigment in the flesh is always accompanied by red skin. A photograph showing the different phenotypes is shown in Figure 1. Although two categories of degree of flesh pigmentation are presented here, the original designation of the *Pf* gene referred to presence and absence of pigment in the flesh without qualification of distribution (De Jong 1987). The results are highly variable in terms of proportion of progeny that display red pigment in the flesh in terms of total percent with any level of pigmented flesh. The degree of pigmentation does not appear to be under simple genetic control. The parents N40-2 and NDOP5847-1 produced variable percentages of completely red progeny depending on the white fleshed parent used (Table 2). A cross between two red-fleshed parents yields proportionately more red-fleshed progeny than one where one parent is red fleshed. Red x red crosses produced almost twice as many red-fleshed progeny than red x white and reciprocals. The proportion of completely red-fleshed progenies in the red x red was more than three times greater than the red x white and reciprocal (Table 3). Although the inheritance of degrees of flesh pigmentation may be influenced by a number of undescribed genes with perhaps minor effects, it is a general rule

TABLE 2—*Segregation of different pigment phenotypes occurring in crosses involving red- and white-fleshed types. Numbers of progeny are accompanied by percentages in parentheses.*

Type	Code	Pistillate Parent	Pollen Parent	Phenotypes			
				RCR[1]	RPR	RW	WW
Red Flesh x Red Flesh							
RCR x RCR1	PA97B23	Cranberry Red	N40-2	16 (21)	32 (43)	10 (13)	17 (23)
RCR selfed	PA97B28	N40-2	N40-2	11 (12)	46 (48)	9 (9)	29 (31)
RCR x RCR	PA97B37	NDOP5847-1	N40-2	22 (20)	61 (55)	17 (15)	11 (10)
RCR x RCR	PA97B39	Cranberry Red	NDOP5847-1	1 (5)	13 (65)	5 (25)	1 (5)
Red Flesh x White Flesh and Reciprocal							
RCR x WW	PA97B33	NDOP5847-1	Andover	1 (8)	3 (23)	6 (46)	3 (23)
RCR x WW	PA97B34	NDOP5847-1	A89875-5	15 (10)	69 (47)	21 (14)	43 (29)
RCR x WW	PA97B35	NDOP5847-1	Pike	3 (3)	50 (43)	53 (45)	11 (9)
RCR x WW	PA97B36	NDOP5847	Kanona	0 (0)	29 (30)	57 (59)	10 (10)
RCR x WW	PA97B38	NDOP5847-1	NZA8904-2	0 (0)	22 (17)	103 (82)	1 (1)
RCR x WW	PA97B40	NDOP5847-1	A8469-5	4 (4)	45 (40)	31 (27)	33 (29)
WW x RCR	PA97B42	AWN86514-2	NDOP5847-1	9 (23)	18 (45)	9 (23)	4 (10)
RCR x WW	PA97B21	Atlantic	N40-2	1 (1)	25 (25)	47 (41)	41 (36)
WW x RCR	PA97B22	Abnaki	N40-2	6 (7)	23 (27)	27 (31)	30 (35)
RCR x WW	PA97B26	N40-2	A89875-5	6 (7)	34 (38)	15 (17)	34 (38)
WW x RCR	PA97B41	A8469-5	N40-2	1 (1)	37 (34)	33 (39)	25 (26)

Phenotypes: RCR = Red skin, completely red flesh; RPR = Red skin, partially red flesh, RW = Red skin, white flesh; WW = White skin, white flesh.

Figure 1. Examples of segregation phenotypes in progeny Upper row shows WW, PW, PPP, and PCP phenotypes (right to left) Lower row shows WW, RW, RPR, and RCR (right to left).

that red-flesh x red-flesh is a more efficient cross type for obtaining larger proportions of red-fleshed progenies.

Two progenies that produced both red- and purple-fleshed segregants are presented in Table 4. Since neither A77715-6 nor the cultivar Serrana are purple-fleshed types and no purple skin or purple skin segregants appeared in other crosses, it is inferred that they harbored the *P* gene. The appearance of purple segregants in these progenies may be explained by the presence of *P* in both progenies epistatically suppressed by a nulliplex or *iiii* genotype at the *I* locus. Crossing to *D-/I-* red-skinned, red-fleshed genotypes gave rise to progenies with *P-/I-* constitution that were purple skinned, and the additional presence of *Pf* was expressed as purple skin, purple flesh (PPP and PCP in Table 4). Reference to Figure 1 serves as a guide to phenotypes of partially and completely purple-fleshed progeny.

TABLE 3—*Averages and ranges (in parentheses) of partially red, completely red, and the sum of the two categories of crosses: red-fleshed x red-fleshed and red-fleshed x white fleshed and reciprocal.*

Cross Type	Percent Completely Red Average (Range)	Percent partiallyRed Average (Range)	Percent of Partially Red and Completly Red Average (Range)	Number of Crosses
Red Flesh x Red Flesh	14.5 (5-20)	52.8 (43-65)	67.2 (60-75)	4
Red Flesh x White Flesh or reciprocal[1]	4.1 (0-10)	32.1 (17-47)	35.2 (17-57)	10

[1] Omitting cross AWN86514-2 x NDOP5847-1 because it deviated significantly in segregation from the rest and was a small sample size.

TABLE 4—*Segregation of different pigment phenotypes occurring in crosses involving red and white flesh types with putative* P-/iiii *genotypes. Numbers of progeny are accompanied by percentages in parentheses.*

				Phenotypes						
Type	Code	Female	Pollen	PCP	PPP	PW	RCR	RPR	RW	WW
RR x WW*	PA97B29	N40-2	A77715-6	2 (3)	11 (17)	7 (11)	1 (2)	10 (16)	30 (30)	2 (3)
WW* x RR	PA97B50	Serrana	Red-fleshed Bulk	11 (7)	34 (20)	12 (7)	0 (0)	17 (10)	27 (16)	67 (40)

Phenotypes: PCP = Purple skin, complete purple flesh; PPP = Purple skin, partially purple flesh; RCR = Red skin, completely red flesh; RPR = Red partially red flesh; RW = Red skin, white flesh; WW = White skin, white flesh, WW* = white flesh phenotype, but *P-/iiii* genotype. Purple pigment appears in progeny in skin and flesh.

TABLE 5—*Total anthocyanin and percentage of three different acylated anthocyanidin glycosides in red- and purple-flesh potatoes.*

Breeding Clone Identification	Total Anthocyanin Content (mg/100 g-FW)[2]	Significance[1]	Flesh Color	Acylated petunidin glycoside (%)[3]	Acylated pelargonidin glycoside (%)[3]	Acylated peonidin glycoside (%)[3]-
PA96RR1-193	12.76	H	Red	-	81.8	-
PA96RR1-220	18.40	DE	Red	-	81.8	-
PA97B21-1	15.32	EFGH	Red	-	81.9	-
PA97B23-12	15.67	EFGH	Red	-	83.4	-
PA97B23-2	13.39	GH	Red	-	81.0	-
PA97B23-3	14.07	FGH	Red	-	84.0	-
PA97B23-4	22.67	BC	Red	-	82.0	-
PA97B28-8	17.23	EF	Red	-	82.0	-
PA97B29-2	12.32	H	Purple	60.6	-	23.9
PA97B29-3	6.86	I	Red	-	79.1	-
PA97B29-4	7.57	I	Purple	64.9	-	20.9
PA97B29-5	5.52	I	Purple	34.2	-	34.8
PA97B29-6	17.09	EFG	Purple	62.1	-	24.8
PA97B35-1	17.42	EF	Red	-	78.8	-
PA97B35-2	21.19	CD	Red	-	79.7	-
PA97B36-3	13.98	FGH	Red	-	74.2	-
PA97B37-2	16.67	EF	Red	-	79.6	-
PA97B37-3	34.96	A	Red	-	85.5	-
PA97B37-7	32.11	A	Red	-	85.8	-
PA97B39-2	25.72	B	Red	-	87.2	-
NDOP5847-1	13.43	GH	Red	-	82.0	-

[1]Monomeric anthocyanin.
[2] Means not sharing the same letter are significantly different ($P < 0.05$) by Duncan's Multiple Range Test.
[3]Percent of total anthocyanin comprised by component.

Total Anthocyanin

The progenies in Tables 2 and 4 were grown in the field for 2 years, first as single hills and the following year as 12-hill plots. Clones selected at the 12-hill stage were analyzed for total anthocyanin content (TA). The TA in the progenies of crosses shows a wide range of expression. The highest levels of expression were found in two progeny from the cross PA97B37 (NDOP5847-1 x N40-2), a red-fleshed x red-fleshed cross (Table 5). The lowest values were found in the cross PA97B29 (N40-2 x A77715-6), a red-fleshed x white fleshed cross. Three progeny of this cross, two purple fleshed and one red fleshed, show statistically lower TA levels than all other clones tested. Purple flesh does not always show a low TA as can be seen by the selection PA97B29-6. The two highest levels of TA were found in clones PA97B37-3, and -7, which averaged five-fold higher than the lowest TA clones (PA97B29-2, -3, and -4). This difference suggests that there may be considerable gain from selecting for higher TA in a breeding population. It further implies that attempts to select higher TA than that found in this study may be successful.

Identity of Anthocyanins

HPLC analysis of the anthocyanin extracts found that the red-fleshed clones have a predominant pigment peak, which corresponds to acylated pelargonidin glycoside. This was identical to the finding of Rodriguez-Saona et al. (1998). Other minor peaks were found, but as can be appreciated in Table 5, a single peak of this anthocyanin type predominated (Figure 2). The red-fleshed clones consisted almost entirely of cinnamic acid acylated derivatives of pelargonidin-3-rutinoside-5-glucoside established by saponification and acid hydrolysis. Saponification produced pelargonidin-3-rutinoside-5-glucoside and ferulic and *p*-coumaric acids. Acid hydrolysis produced mainly the aglycon pelargonidin. After similar processing the purple clones were found to be primarily acylated derivatives of petunidin-3-rutinoside-5-glucoside and peonidin-3-rutinoside-5 glucoside (Figure 2, panel B, peaks 2 and 3, respectively) with the former predominating in three out of four clones (Table 5). There is evidence that the acylating acids were ferulic and *p*-coumaric acids. In addition to petunidin and peonidin, acid hydrolysis yielded minor amounts of malvidin

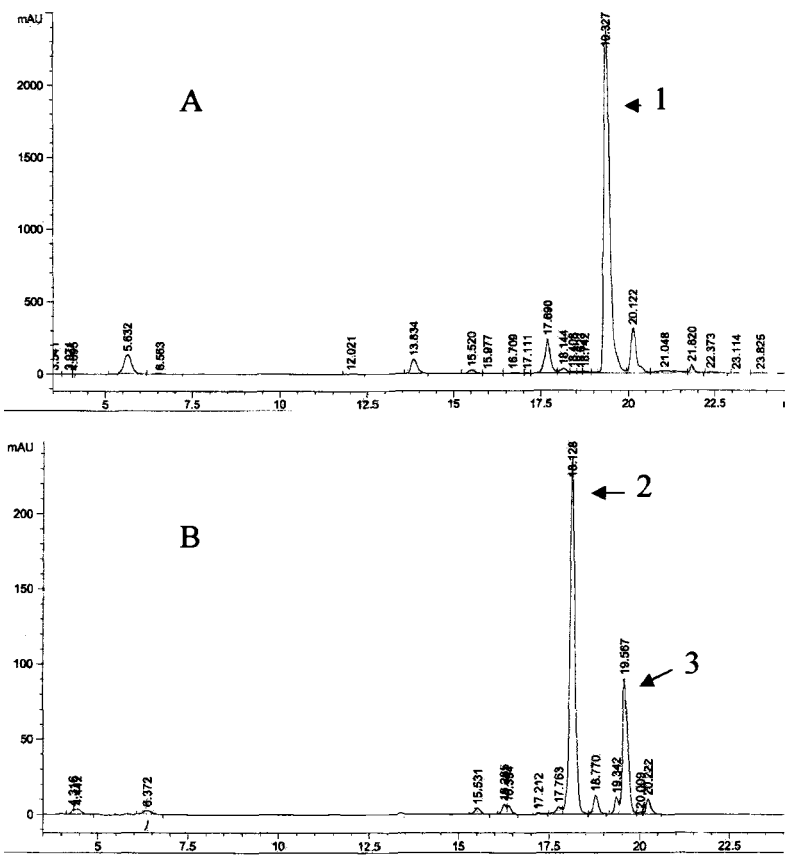

Figure 2.
HPLC separation of anthocyanin extracts from red-fleshed (A) and purple-fleshed potatoes (B). Peak 1 labeled in panel A, was identified by saponification, hydrolysis and HPLC analysis to be an acylated pelargonidin glycoside. Peaks 2 and 3 in panel B were identified by the same means as acylated glycosides of petunidin and peonidin, respectively.

TABLE 6—*Antioxidant potential in red, purple, yellow-orange, and white fleshed potato as measured by Oxygen Radical Absorbance Capacity (ORAC) and Ferrous Radical Absorbance Plasma (FRAP).*

Identification	Flesh Color	ORAC[1]	FRAP[1]
PA97B23-2	Red	14.5[2] c	9.02 c
PA97B23-4	Red	22.0 b	12.1 a
NDOP5847-1	Red	25.0 a	10.7 b
PA97B29-4	Purple	15.7 c	9.6 bc
PA97B29-5	Purple	21.3 b	12.5 a
91E22	Yellow-Orange	11.5 d	5.7 d
Reddale	White	8.1 e	3.7 e

[1]Means are significantly different at the $P < 0.05$ level in the Duncan's Multiple Range Test.
[2] microM Trolox units per gram fresh weight of tuber.

and cyanidin. The three sources of red-fleshed trait in this study have no known genetic relationship that suggests that the finding of a single aglycon indicates that this may be general feature of this trait in red-fleshed potato.

Antioxidant Values

Both measures of antioxidant activity (ORAC and FRAP) showed that the red- and/or purple-fleshed potatoes had significantly higher antioxidant values than the white- or yellow/orange-fleshed potatoes (Table 6). These values indicated that the high anthocyanin potatoes ranged up to a threefold increase in antioxidant activities. Although the yellow/orange-fleshed potato is known to have a higher level of zeaxanthin, a xanthophyll known to function as an antioxidant, the anthocyanin extraction method applied would not be expected to effectively extract lipophilic carotenoids nor would the aqueous buffer be a satisfactory solvent for these in the two antioxidant tests. The ORAC and FRAP values were closely correlated ($r = .90$, $P < .001$).

CONCLUSIONS

This study has demonstrated the feasibility of selecting potato clones with high levels of anthocyanins resulting in red- and purple-fleshed potatoes. Solidly pigmented progeny can be obtained by crossing pigmented and unpigmented parents, but greater percentage recovery of pigmented flesh types occurs by crossing of two pigmented flesh types. Purple-fleshed progeny were obtained from two crosses that did not involve a purple-fleshed parent presumably due to the co-presence of an unexpressed *P* gene and the suppressor *I* in the white-fleshed parents.

Anthocyanin levels ranged greatly from 5.5 to 35.0 mg/ 100 g FW. The anthocyanins were of several types, acylated glycosides of pelargonidin in the red-fleshed clones while the purple-fleshed clones contained larger peaks of acylated glycosides of petunidin and peonidin and smaller peaks of malvidin and cyanidin. Antioxidant measurements indicated that these potatoes may present the possibility of enhancing the nutritional contribution of potatoes to the antioxidants in the human diet. Potato genotypes may be developed through breeding that would place potato into the category of foods that are eaten for their antioxidant properties.

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